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Research paper

PEG-OCL micelles for quercetin solubilization and inhibition of cancer cell growth

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ABSTRACT

In this study, quercetin (QCT), a flavonoid with high anticancer potential, was loaded into polymeric micelles of PEG-OCL (poly(ethylene glycol)-*b*-oligo(ε-caprolactone)) with naphthyl or benzyl end groups in order to increase its aqueous solubility. The cytostatic activity of the QCT-loaded micelles toward different human cancer cell lines and normal cells was investigated. The results showed that the solubility of QCT entrapped in mPEG750-*b*-OCL micelles was substantially increased up to 1 mg/ml, which is approximately 110 times higher than that of its solubility in water (9 μg/ml). The average particle size of QCT-loaded micelles ranged from 14 to 19 nm. The QCT loading capacity of the polymeric micelles with naphthyl groups was higher than that with benzyl groups (10% and 6%, respectively). QCT-loaded, benzyl- and naphthyl-modified micelles effectively inhibited the growth of both sensitive and resistance cancer cells (human erythromyelogenous leukemia cells (K562) and small lung carcinoma cells (GLC4)). However, the benzyl-modified micelles have a good cytocompatibility (in the concentration range investigated (up to 100 μg/ml), they are well tolerated by living cells), whereas their naphthyl counterparts showed some cytotoxicity at higher concentrations (60–100 μg/ml). Flow cytometry demonstrated that the mechanism underlying the growth inhibitory effect of QCT in its free form was inducing cell cycle arrest at the G2/M phase. Benzyl-modified micelles loaded with QCT also exhibited this cycle arresting the effect of cancer cells. In conclusion, this paper shows the enhancement of solubility and cell cycle arrest of QCT loaded into micelles composed of mPEG750-*b*-OCL modified with benzyl end groups. These micelles are therefore considered to be an attractive vehicle for the (targeted) delivery of QCT to tumors.

1. Introduction

Many potent bioactive anticancer compounds are rather hydrophobic and consequently have a low aqueous solubility, which generally leads to a low bioavailability. Many reports have shown that drug delivery systems can improve the bioavailability of such compounds as well as prevent drug degradation and increase drug concentration at the target area [1–3]. One approach to solubilize poorly soluble drugs is to load them in the hydrophobic core of surfactant micelles [4,5] that are formed above the critical micelle concentration (CMC). However, the CMC of classical surfactants is relatively high resulting, after administration and subsequent dilution in the blood stream, in rapid destabilization. During the last two decades, a new class of micelles has been designed which are based on amphiphilic block copolymers. The polymers are characterized by a very low CMC (10^{-6}–10^{-7} M, which is about 1000 times lower than that of classical surfactant micelles) and form micelles with a size of 100 nm or less [6,7]. Therefore, polymeric micelles are presently investigated for the solubilization of hydrophobic drugs and, because of their small size, for targeted delivery, exploiting the so-called the enhanced permeability and retention (EPR) effect [8–13].

Quercetin (QCT) is a flavonoid compound that is present in different fruit and vegetable species. It has been reported that QCT has effects on cancer chemoprevention, likely because of its antioxidant activity [14,15]. QCT has also shown to have cytotoxic effects on cancer cells/tumors through several other mechanisms, such as cell cycle arrest, induction of apoptosis, and inhibition of angiogenesis [16–20]. Despite these interesting biological activities, its low aqueous solubility hampers its use as a therapeutic agent [21,22]. The aims of the present study were to load QCT into polymeric micelles in order to increase aqueous solubility and to study the cytostatic activity of the loaded micelles toward two different cancer cells. For solubilization, we used end-group-modified amphiphilic oligomers based on PEG-OCL which are characterized by a low CMC and a high loading capacity of hydrophobic compounds [23–25].

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2. Materials and methods

2.1. Materials

Propidium iodide (PI) was purchased from US Biological (Massachusetts, USA). QCT, Triton X-100, Ribonuclease A (RNase), Histopaque®-1077, and 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS) was obtained from Biochrom AG (Berlin, Germany). RPMI 1640, trypsin–EDTA, and penicillin–streptomycin were purchased from GIBCO™ Invitrogen (Grand Island, NY, USA). Dichloromethane, dimethyl sulfoxide (DMSO), ethanol, and Tween 80 were purchased from Merck (Darmstadt, Germany). All other chemicals were of the highest grade available. Methoxy-poly(ethylene glycol)-*b*-oligo(ε-caprolactone) (mPEG-*b*-OCL) block oligomers with different end groups (Fig. 1) were prepared as described previously [23]. Briefly, mPEG-*b*-OCL was synthesized by ring-opening polymerization of ε-caprolactone (175 mmol), initiated by mPEG750 (35 mmol) and catalyzed by $SnOct_2$ (1.8 mmol) overnight at a temperature of 130 °C. Benzoylated and naphthoylated mPEG750-*b*-OCL (mPEG750-*b*-OCL-Bz, mPEG750-*b*-OCL-Np, respectively) (Fig. 1b and c) were obtained by reacting the hydroxyl end group with a fivefold excess of benzoyl- or 2-naphthoyl-chloride in the presence of an equimolar amount of triethyl amine as HCl scavenger. Then, the end-group-modified copolymers were purified by preparative reversed-phase HPLC (RP-HPLC). The degree of polymerization of the caprolactone block ranged from 2 to 7 units.

2.2. Preparation of QCT-loaded polymeric micelles

Two different amphiphilic block oligomers, mPEG750-*b*-OCL-Bz (Fig. 1b) and mPEG750-*b*-OCL-Np (Fig. 1c), were used to load QCT using the film hydration method described by Carstens et al. [24]. Briefly, QCT was dissolved in ethanol at a concentration of 5 mg/ml, and the oligomers were dissolved in dichloromethane at a concentration of 10 mg/ml. Next, 1 ml of oligomer solution and 0.1, 0.2, 0.4, and 0.8 ml of QCT solution were mixed and transferred into a 10-ml round bottomed flask yielding feed ratios of QCT/oligomer of 5%, 10%, 20%, and 40% (w/w), respectively. A film was obtained after evaporation of the solvents and was subsequently dried for 30 min under an N_2 stream. Then, the resulting film was hydrated with 1 ml of PBS at room temperature. The non-entrapped (precipitated) QCT was removed by filtration through a 0.2-μm filter (nylon syringe filter, Filtrex, USA).

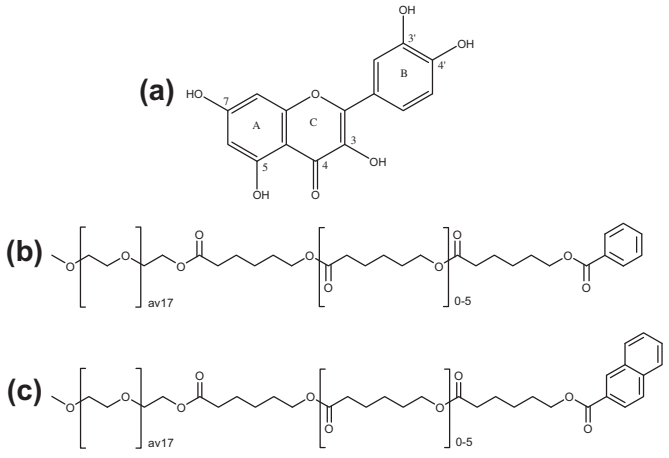

Fig. 1. Chemical structures of QCT (a), benzoylated mPEG750-*b*-$OCL_{2\text{-}7}$ (b), and naphthoylated mPEG750-*b*-$OCL_{2\text{-}7}$ (c).

2.3. Characterization of the QCT-loaded micelles

2.3.1. Loading efficiency and solubility study

The amount of QCT loaded into the micelles was determined by diluting the micellar dispersions with dimethylformamide (DMF) and measuring the absorbance at 372 nm using an UV–vis spectrophotometer. The entrapment efficiency (EE) and loading capacity (LC) were calculated as follows: EE (% w/w) = (amount of loaded QCT/amount of QCT used for loading) × 100% and LC (% w/w) = (amount of loaded QCT/amount of copolymer used for loading) × 100%. The solubility of QCT-loaded micelles (w/v) is defined as the amount of loaded QCT/volume of PBS.

The solubility of free QCT in PBS (pH 7.4) was determined as described in a previous study [24] with some modification. Briefly, an excess amount of QCT was added to 10 ml of PBS. The mixture was stirred at 37 °C for 48 h. Then, the mixture was filtered through a 0.45-μm filter (nylon syringe filter, Filtrex, USA) to remove insoluble QCT. The amount of soluble QCT was measured by UV–vis spectrophotometry at 372 nm. (The extinction coefficient of QCT at this wavelength is 19,487 M^{-1} cm^{-1} [26].)

2.3.2. Dynamic light scattering

The Z_{ave} particle size of loaded and unloaded polymeric micelles was measured by dynamic light scattering (DLS), using a Malvern system (Zetasizer®, version 5.00, Malvern Instruments Ltd., Malvern, UK) consisting of computerized auto-titrate and DLS software. The sizing measurements were taken at a fixed angle of 173°.

2.3.3. Release of QCT from loaded micelles

The *in vitro* release of QCT from micelles was determined by a dialysis method. An aliquot of 1 ml of a freshly prepared micellar dispersion was introduced into a pre-swollen dialysis bag with a molecular weight cutoff at 12,000 Da (Cellu Sep® T4 regenerated cellulose tubular membrane, Membrane Filtration Products, Inc.). The dialysis bag was tightly closed and immersed into 100 ml of 0.2% Tween 80 in PBS at 37 °C for 48 h with gentle stirring at 500 rpm. Samples (10 ml) of the receiving medium were drawn periodically, and the volume was adjusted with fresh buffer containing 0.2% Tween 80. The concentration of QCT in the different samples was measured spectrophotometrically at 372 nm using an UV–vis spectrophotometer (UV-2540, Shimadzu). Moreover, the effect of Tween on micellar stability was studied by DLS.

2.3.4. Cytotoxicity of QCT-loaded micelles on cancer cells

Human erythromyelogenous leukemia cells (K562), the corresponding drug-resistant cells with P-glycoprotein (P-gp) overexpression (K562/ADR), small lung carcinoma cells (GLC4), and the corresponding drug-resistant cells with multidrug resistance-associated protein (MRP1) overexpression (GLC4/ADR) were cultured in RPMI 1640 supplemented with 10% FBS, 100 U/ml of penicillin, and 0.1 mg/ml of streptomycin. The cells were grown in a humidified atmosphere at 37 °C in 5% CO_2. Cell viability was determined by using the trypan-blue dye exclusion method [27]. Cell counts were determined by using a hemocytometer chamber.

Four different types of cancer cell lines (K562, K562/ADR, GLC4, and GLC4/ADR) were used to evaluate the cell inhibitory activity of the QCT in its free form and loaded in the micelles. The inhibition of growth of cells that were incubated with different QCT formulations was tested by counting the cell number via flow cytometer. Briefly, the cells suspended in 1 ml of medium were seeded into the 24-well plates at a density of 5×10^4 cells/well. After 24-h incubation, solutions with different concentrations of QCT-loaded micelles (final concentration of QCT ranged from 0.5 to 10 μg/ml) were added and the cells were further cultured for 72 h. The empty polymeric micelles were also tested at final concentration ranging from 5 to 100 μg/ml. The number of cells was counted by flow

cytometer (Coulter® Epics® XL™), and the inhibition of cell growth was calculated as follows: % cell growth inhibition = $(C_{c\text{-}72h} - C_{s\text{-}72h})/(C_{c\text{-}72h} - C_{c\text{-}0h}) \times 100\%$, where $C_{c\text{-}72h}$ and $C_{c\text{-}0h}$ represent the number of viable cells in control samples at 72 and 0 h, respectively, and $C_{s\text{-}72h}$ represents the number of viable cells in samples exposed to QCT at 72 h. The 50% inhibitory concentration (IC_{50}) was determined from the dose–response curves of percentages of cell growth inhibition versus QCT concentration.

2.3.5. Cytotoxicity of QCT-loaded micelles on normal cells

Peripheral blood mononuclear cells (PBMCs) isolated from freshly drawn blood of a human volunteer were diluted at a 1:1 volume ratio with PBS and anticoagulated with heparin. The blood sample was subsequently layered onto Histopaque®-1077 at a volume ratio of 3:1 and centrifuged at 1500*g* for 30 min. With this procedure, the PBMCs were separated from the erythrocytes, granulocytes, and platelets. The PBMCs layer was removed and then washed twice with PBS. The cells were resuspended in RPMI 1640 culture medium supplemented with 10% FBS, 100 U/ml of penicillin, and 0.1 mg/ml of streptomycin. Cell viability was determined by using the trypan-blue dye exclusion method [27]. Cell counts were determined using a hemocytometer chamber.

The cytotoxicity of the QCT in its free form and loaded in polymeric micelles as well as empty polymeric micelles toward PBMCs was tested by using a standard MTT assay as described by Alley et al. [28], with some modification. Briefly, 100 μl of PBMCs suspended in cell growth medium was pipetted into each well of 96-well tissue culture plates. After 24-h incubation, dispersions with different concentrations of QCT-loaded polymeric micelles (final concentration of QCT ranged from 0.5 to 10 μg/ml) were added and the cells were further incubated for 48 h. The empty polymeric micelles were also tested (final concentration of polymer ranged from 5 to 100 μg/ml). Next, 100 μl of the medium was removed, and 15 μl of 5 mg/ml MTT dye in PBS was added to the wells. Plates were incubated at 37 °C for 4 h in humidified 5% CO_2 atmosphere. After that, the MTT solution was removed, and 200 μl of DMSO was added and mixed thoroughly to dissolve the dye crystals. The absorbance at 540 nm was measured using a microtiter plate reader with a reference wavelength of 630 nm. The cell viability was determined as follows: % cell viability = (mean absorbance in test wells)/(mean absorbance in control wells) × 100%.

2.3.6. Effect of QCT-loaded micelles on cancer cell cycle progression

Cells were exposed to different formulations (10 μg/ml QCT, 10 μg/ml QCT-loaded mPEG750-*b*-OCL-Bz micelles, and 100 μg/ml empty mPEG750-*b*-OCL-Bz micelles) at 37 °C for 5 min–72 h and subsequently washed by PBS. Next, the cells were fixed overnight with 70% ethanol, subsequently washed with PBS, and stained for 30 min in the dark at 37 °C with propidium iodide (PI) in PBS (10 μg/ml), also containing RNase (0.2 mg/ml) and 0.1% of Triton X-100. The cell cycle progression and cellular kinetics were analyzed by flow cytometer (Coulter® Epics® XL™), and the data were analyzed by the origin software program. The number of cells in different cell cycle phases (G0/G1, S, G2/M) was calculated using DNA content histograms [29].

2.4. Statistical analysis

The experiments were done in the triplicate, and the results are expressed as mean ± SD. Statistical analysis was done by using one-way ANOVA and *p*-value at a level of 95% confidence limit.

3. Results and discussion

3.1. Loading of QCT into polymeric micelles

Amphiphilic oligomers based on caprolactone and PEG with either benzoyl (–Bz) and naphthoyl (–Np) end groups, mPEG750-*b*-OCL-Bz (Fig. 1b), and mPEG750-*b*-OCL-Np (Fig. 1c), respectively, were investigated for their solubilization capacity of a hydrophobic compound (Quercetin, QCT, Fig. 1a). In a previous study, it was demonstrated that mPEG-*b*-OCL micelles without benzyl and naphthyl groups were very unstable and had a low drug-loading capacity [24]. Therefore, in the present study, unmodified mPEG-*b*-OCL micelles were excluded. The results (Table 1) reveal that QCT was successfully loaded into both micellar systems prepared using a film hydration method. It was found that the QCT-loaded micellar dispersions have a clear yellow appearance (Fig. 2a and b). It was shown that the micelles were stable and did not leach QCT after incubation at room temperature for more than 1 month. Table 1 also shows that at high polymer/drug ratio, QCT was quantitatively entrapped. As expected, with increasing drug to polymer ratio in the feed, the encapsulation efficiency decreased. The loading efficiency reached a maximum of about 6% and 10% for the mPEG750-*b*-OCL-Bz and mPEG750-*b*-OCL-Np micelles, respectively, indicating that their cores were saturated with QCT. The good loading capacity of the micelles can likely be ascribed by a combination of hydrophobic interactions between QCT and the OCL chains as well as due to π–π stacking of QCT and the aromatic capping groups. The loading capacity of QCT-loaded mPEG750-*b*-OCL-Np micelles (ranging from 4.5% to 10.2%) was higher than that of mPEG750-*b*-OCL-Bz (ranging from 4.0% to 6.0%), which might be ascribed to the better solubilizing properties of the more bulky naphthyl group. Molavi et al. [8] also have reported that the presence of aromatic rings in poly(ε-caprolactone) blocks increased the compatibility with a hydrophobic drug. QCT-loaded mPEG750-*b*-OCL-Np micelles were solubilized in PBS up to 1.02 ± 0.06 mg/ml, which is significantly (p-value < 0.05) higher than that of QCT-loaded mPEG750-*b*-OCL-Bz micelles (0.60 ± 0.09 mg/ml), while the solubility of free QCT in PBS is only 9.04 ± 0.18 μg/ml. This result indicates that the aqueous solubility of QCT entrapped in mPEG750-*b*-OCL micelles significantly increased with a factor of

Table 1
Loading of QCT in polymeric micelles composed of mPEG750-*b*-OCL with benzyl and naphthyl capping groups.

Polymer	Polymer/QCT ratio (w/w)	EE (%)	LC (%)	Z_{ave} (nm)	PDI
mPEG750-*b*-OCL-Bz	10:0	–	–	15.6 ± 1.3	0.2 ± 0.1
	20:1	102.6 ± 2.8	5.1 ± 0.1	18.7 ± 1.8	0.3 ± 0.0
	10:1	59.5 ± 8.9	6.0 ± 0.9	17.8 ± 0.6	0.2 ± 0.0
	10:2	25.4 ± 14.5	5.1 ± 2.9	15.9 ± 1.7	0.2 ± 0.0
	10:4	7.9 ± 0.5	4.0 ± 0.3	16.7 ± 4.8	0.1 ± 0.0
mPEG750-*b*-OCL-Np	10:0	–	–	14.9 ± 0.2	0.2 ± 0.0
	20:1	89.8 ± 16.6	4.5 ± 0.8	13.8 ± 1.3	0.3 ± 0.0
	10:1	102.4 ± 6.3	10.2 ± 0.6	16.6 ± 1.7	0.2 ± 0.0
	10:2	34.1 ± 11.0	6.8 ± 2.2	17.0 ± 1.7	0.3 ± 0.1
	10:4	13.1 ± 0.3	5.2 ± 0.1	18.0 ± 0.7	0.2 ± 0.1

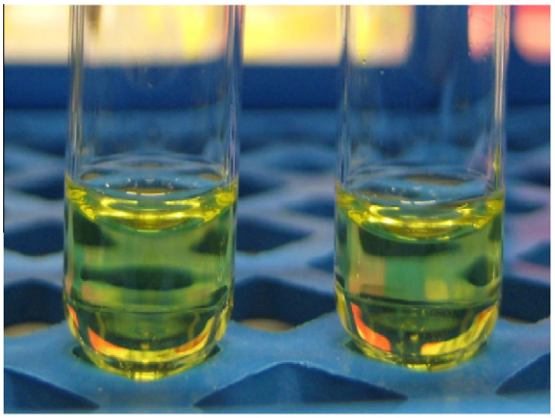

Fig. 2. mPEG-*b*-OCL-Bz (a) and mPEG-*b*-OCL-Np (b) polymeric micellar dispersions loaded with QCT at drug and polymer concentrations of 1 and 10 mg/ml, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

~70–110. The results also demonstrate that micelles, independent of the polymer capping group and independent of their QCT loading, had a size between 14 and 19 nm with a low polydispersity index of average of <0.2, in agreement with previous reports [23,24]. Particulate drug carriers of a size range of 10–100 nm can passively accumulate in tumors area via the EPR effect [30–32]. Particles with a size around 10 nm might also pass the leaky vasculature of the liver. However, when the particles are not recognized by hepatocytes, they are not retained in the liver and re-enter the circulation [33]. To further substantiate this, Lukyanov et al. [34] reported that micelles with a size ranging from 7 nm to 35 nm showed effective tumor accumulation *in vivo*.

The CMC of micelles composed of end-group-modified OCL-PEG amphiphiles is as low as 0.003 mg/ml [23], whereas the CMC of normal PEG-PCL micelles is around 0.03–0.3 mg/ml [35,36]. The latter micelles were relatively stable as evidenced from their blood circulation half-life (around 30 h [36]). So, also a good *in vivo* stability of the micelles based on end-group-modified OCL-PEG amphiphiles can be expected making them suitable for targeting to tumors exploiting the EPR effect.

3.2. Release of QCT from loaded micelles

The *in vitro* release of QCT from loaded mPEG750-*b*-OCL-Bz and mPEG750-*b*-OCL-Np micelles (1 mg of QCT, 10 mg of polymer per 1 ml) was determined by a dialysis method (see Section 2.3.3). Tween was added to the external solution to solubilize released QCT and thus to maintain sink conditions. DLS analysis as shown in Fig. 3 exhibited two separate peaks of mPEG750-*b*-OCL micelles and Tween, demonstrating that Tween did not destabilize the mPEG750-*b*-OCL micelles. Fig. 4 shows that after 1- to 2-h incubation, QCT started to release from the micelles. This delay is probably due to the permeation of QCT through the dialysis membrane. In the next hour, 30 ± 5% of QCT was released from the naphthyl micelles, whereas approximately 64 ± 5% was released from the benzyl micelles. QCT was subsequently slowly released the following 20 h and reaching 73 ± 10% and 89 ± 9% from the micelles with naphthyl and benzyl groups, respectively. It can be concluded that the micelles with the naphthyl groups released QCT slower than those with the benzyl groups. This can be ascribed to a stronger interaction of QCT with naphthyl groups than that with benzyl groups.

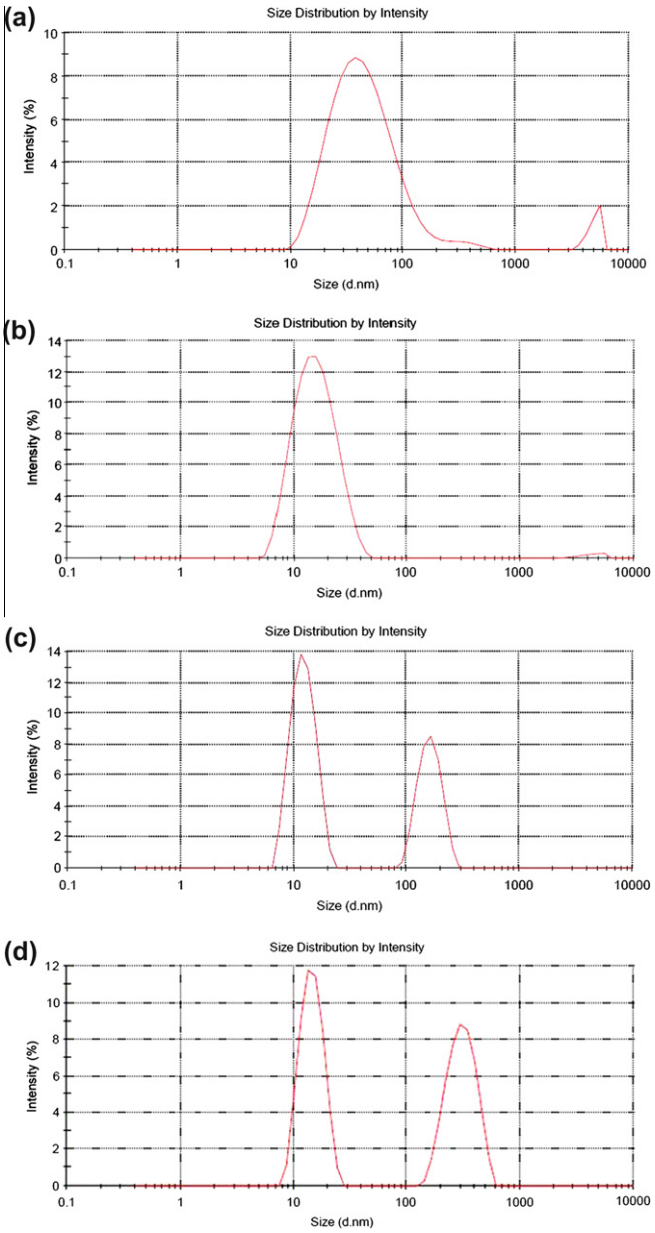

Fig. 3. Size distribution as measured by DLS of QCT-loaded mPEG750-*b*-OCL-Bz micelles (a), 0.2% Tween (b), the mixture of QCT-loaded mPEG750-*b*-OCL-Bz micelles and 0.2% Tween at 1 h (c) and 48 h (d). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Cytotoxicity of QCT-loaded micelles on cancer cells

The cytotoxicity results of sensitive and resistant cancer cell lines incubated with QCT-loaded mPEG750-*b*-OCL micelles and empty polymeric micelles are shown in Figs. 5 and 6, respectively. The concentrations of micelles causing 50% of cell growth inhibition (IC_{50} values) are summarized in Table 2. It was found that QCT-loaded mPEG750-*b*-OCL-Bz micelles were effective to inhibit the growth of leukemia K562 cells and small lung cancer GLC4 cells with IC_{50} values of 10.0 ± 0.1 and 6.8 ± 0.9 μg/ml, respectively. These micelles also showed cell growth inhibition activity against resistance leukemia (K562/ADR) cells and small lung cancer (GLC4/ADR) cells with the IC_{50} values of 7.8 ± 0.1 and 5.7 ± 0.9 μg/ml, respectively. The QCT-loaded mPEG750-*b*-OCL-Np micelles had a significantly (p-value < 0.01) higher antiproliferative

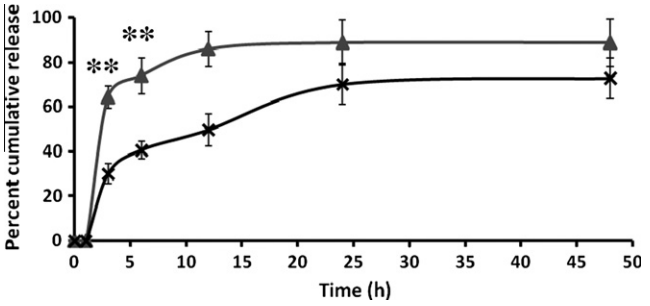

Fig. 4. Release of QCT from mPEG750-*b*-OCL-Np (×) and mPEG750-*b*-OCL-Bz micelles (▲), pH 7.4, 37 °C. Statistical significance: $^{**}p$-value < 0.05.

activity against all tested cancer cells than the benzyl micelle with IC_{50} values ranging from 2.4 ± 0.1 to 4.3 ± 0.4 μg/ml. To clarify this, the cytotoxicity of the empty micelles was also investigated. It was found that the empty mPEG750-*b*-OCL-Np micelles (5–100 μg/ml) demonstrated cytotoxicity toward the studied cancer cell lines with IC_{50} values ranging from 41.0 ± 1.6 to 94.2 ± 5.6 μg/ml. On the other hand, the empty mPEG750-*b*-OCL-Bz micelles showed cell growth inhibition of less than 10% at 100 μg/ml. These findings are in agreement with a previous report [24], in which it was shown that mPEG750-*b*-OCL-Bz micelles had a good cytocompatibility, while the corresponding naphthyl micelles showed some toxicity. It can therefore be concluded that the greater antiproliferative effect of QCT-loaded mPEG750-*b*-OCL-Np is due to a combined effect of QCT and the mPEG750-*b*-OCL-Np copolymer.

Free QCT had potent cytotoxicity toward leukemia K562 cells and small lung cancer GLC4 cells with IC_{50} values of 6.7 ± 0.3 μg/ml and 6.9 ± 0.6 μg/ml, respectively. Free QCT had lower (p-value < 0.05) cytotoxicity toward resistant K562/ADR (8.3 ± 0.7 μg/ml) than sensitive K562 cells but higher cytotoxicity toward resistant GLC4/ADR (4.8 ± 1.2 μg/ml) than sensitive GLC4 cells. Interestingly, QCT-loaded polymeric micelles had a higher cytotoxicity toward resistant K562/ADR and GLC4/ADR cell lines than toward the sensitive cell lines (K562 and GLC4, respectively), especially, QCT-loaded, benzyl ending micelles that had significantly (p-value < 0.01) higher cytotoxicity toward resistant K562/ADR than that of its corresponding sensitive cell (10.0 ± 0.1 μg/ml and 7.8 ± 0.1 μg/ml, respectively). This higher cytotoxicity of QCT loaded in benzyl ending mPEG750-*b*-OCL micelles toward resistant cancer cells might be due to the uptake of the micelles by the cells and subsequent intracellular QCT release. Moreover, the substantial higher cytotoxicity of mPEG750-*b*-OCL micelles toward resistant cells might be due to intracellular depletion of ATP due to the amphiphilic OCL-PEG oligomer as previously observed for pluronic block copolymers [37–39]. But this hypothesis needs further investigation.

3.4. Cytotoxicity of QCT-loaded micelles on normal cells

The MTT assay was used to access the safety of QCT-loaded micelles as well as empty micelles and free QCT on 'normal' cells represented by the peripheral blood mononuclear cells (PBMCs). Fig. 7 shows that free QCT and QCT-loaded mPEG750-*b*-OCL-Bz micelles had no cytotoxic effect on PBMCs upto 10 μg/ml (QCT) and 100 μg/ml (micelles), whereas the toxicity toward PBMCs was found with QCT-loaded mPEG750-*b*-OCL-Np micelles at high concentration of 10 μg QCT/ml (55.8 ± 1.5% cell growth inhibition). Similar toxicity was found by the empty mPEG750-*b*-OCL-Np micelles (100 μg/ml), demonstrating again a better cytocompatibility of the empty mPEG750-*b*-OCL-Bz micelles.

3.5. Effect of QCT-loaded micelles on cancer cell cycle progression

Because of the good cytocompatibility of mPEG750-*b*-OCL-Bz micelles, these particles loaded with QCT were selected for further studies on the mechanism of the growth inhibition of leukemic K562 cancer cells. Flow cytometry analysis with propidium iodide (PI)-stained cells was performed to determine the effect of QCT

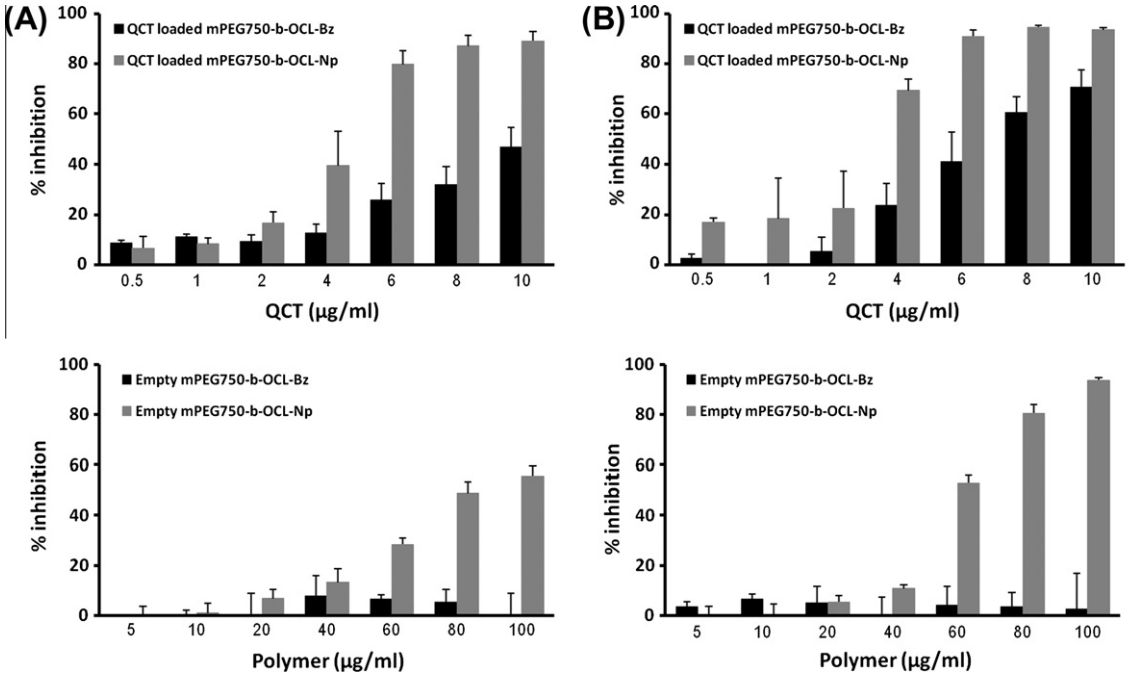

Fig. 5. Cytotoxicity of QCT-loaded mPEG750-*b*-OCL-Bz micelles, QCT-loaded mPEG750-*b*-OCL-Np micelles, empty mPEG750-*b*-OCL-Bz, and empty mPEG750-*b*-OCL-Np copolymer micelles against K562 (A) and GLC4 cells (B). The cell growth inhibition of was performed by counting the number of cells via flow cytometer after 72 h of incubation.

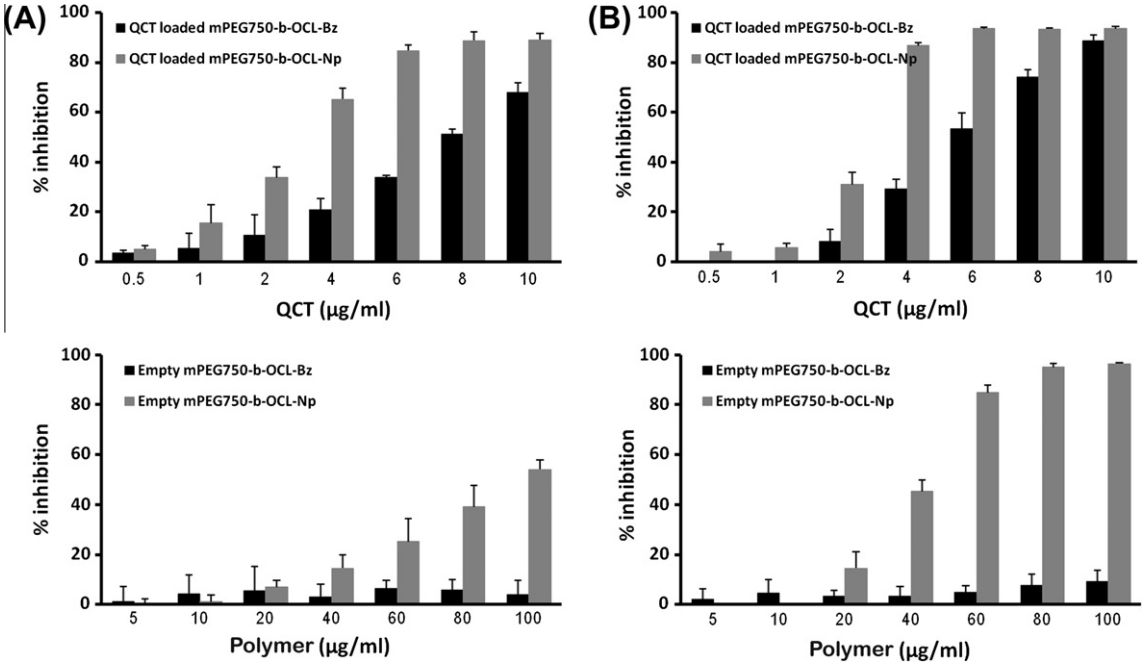

Fig. 6. Cytotoxicity of QCT-loaded mPEG750-*b*-OCL-Bz micelles, QCT-loaded mPEG750-*b*-OCL-Np micelles, empty mPEG750-*b*-OCL-Bz, and empty mPEG750-*b*-OCL-Np copolymer against K562/ADR (A) and GLC4/ADR cells (B). The cell growth inhibition was performed by counting the number of cells via flow cytometer after 72 h of incubation.

Table 2
IC_{50} values of QCT and QCT-loaded mPEG750-*b*-OCL micelles against K562, K562/ADR, GLC4, and GLC4/ADR cell lines; the cell growth inhibition was performed by counting the number of cells via flow cytometer after 72 h of incubation.

Cell lines	IC_{50} (μg/ml)			
	K562	K562/ADR	GLC4	GLC4/ADR
QCT-loaded mPEG-*b*-OCL-Bz	10.0 ± 0.1	7.8 ± 0.1	6.8 ± 0.9	5.7 ± 0.9
QCT-loaded mPEG-*b*-OCL-Np	4.3 ± 0.4	2.9 ± 0.3	3.0 ± 0.7	2.4 ± 0.1
Empty mPEG-*b*-OCL-Bz	ND	ND	ND	ND
Empty mPEG-*b*-OCL-Np	87.5 ± 1.0	94.2 ± 5.6	59.8 ± 1.3	41.0 ± 1.6
Free QCT	6.7 ± 0.3	8.3 ± 0.7	6.9 ± 0.6	4.8 ± 1.2

ND = no toxicity detectable.

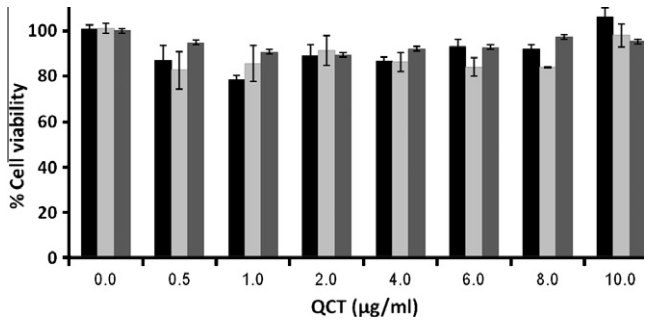

Fig. 7. Cytotoxicity of free QCT (■), QCT-loaded mPEG750-*b*-OCL-Bz polymeric micelles (▒), and empty mPEG750-*b*-OCL-Bz copolymer micelles (▓) toward PBMCs. The cell viability of the different samples was tested using the MTT assay after 48 h of incubation.

both in its free form and loaded in mPEG750-*b*-OCL-Bz micelles. Table 3 presents that the G2/M population of K562 leukemia treated with QCT gradually increased in time (from 21 ± 3% at 5 min to 42 ± 1% at 72 h), while the G0/G1 population decreased (from 54 ± 3% at 5 min to 36 ± 2% at 72 h); G0/G1, S, and G2/M phases of untreated control cells were constant in time with ratios around 56:25:19, respectively. The number of K562 cells in G2/M phase increased from 23 ± 3% at 5 min to 45 ± 1% at 72 h cells upon incubation with QCT-loaded mPEG750-*b*-OCL-Bz micelles. The G0/G1 population decreased from 54 ± 3% to 30 ± 3%. The results show that K562 cells were arrested in the G2/M phase by QCT both in its free form and entrapped in micelles. It is interesting that at G2/M phase, the potential on cell cycle arrest of QCT-loaded mPEG750-*b*-OCL-Bz micelles was slightly higher (p-value < 0.01) than that of free QCT, particularly at 72 h of incubation as shown in Fig. 8. It was further demonstrated that the cellular population at G0/G1, S, and G2/M phases of cells treated with empty mPEG750-*b*-OCL-Bz micelles was constant. The same result was

Table 3
K562 cell cycle distribution.

Treatment period		Cell cycle distribution		
		G0/G1	S	G2/M
5 min	Control	55.9 ± 1.6	24.6 ± 1.4	19.5 ± 2.8
	QCT	53.6 ± 3.2	25.4 ± 0.1	21.0 ± 3.3
	QCT-loaded micelles	54.5 ± 2.6	22.7 ± 0.7	22.8 ± 3.2
24 h	Control	56.0 ± 1.0	24.9 ± 0.6	19.2 ± 1.5
	QCT	40.3 ± 2.3	24.0 ± 0.4	35.7 ± 1.9
	QCT-loaded micelles	41.9 ± 3.6	23.3 ± 1.0	34.8 ± 2.7
48 h	Control	57.0 ± 0.8	25.8 ± 2.0	17.2 ± 2.8
	QCT	37.1 ± 2.9	23.6 ± 2.0	39.3 ± 1.0
	QCT-loaded micelles	32.3 ± 4.7	26.0 ± 0.6	41.8 ± 4.8
72 h	Control	57.1 ± 0.5	24.2 ± 0.8	18.7 ± 1.3
	QCT	35.6 ± 2.0	23.0 ± 1.0	41.5 ± 0.8
	QCT-loaded micelles	29.5 ± 3.0	25.9 ± 1.8	44.7 ± 0.9

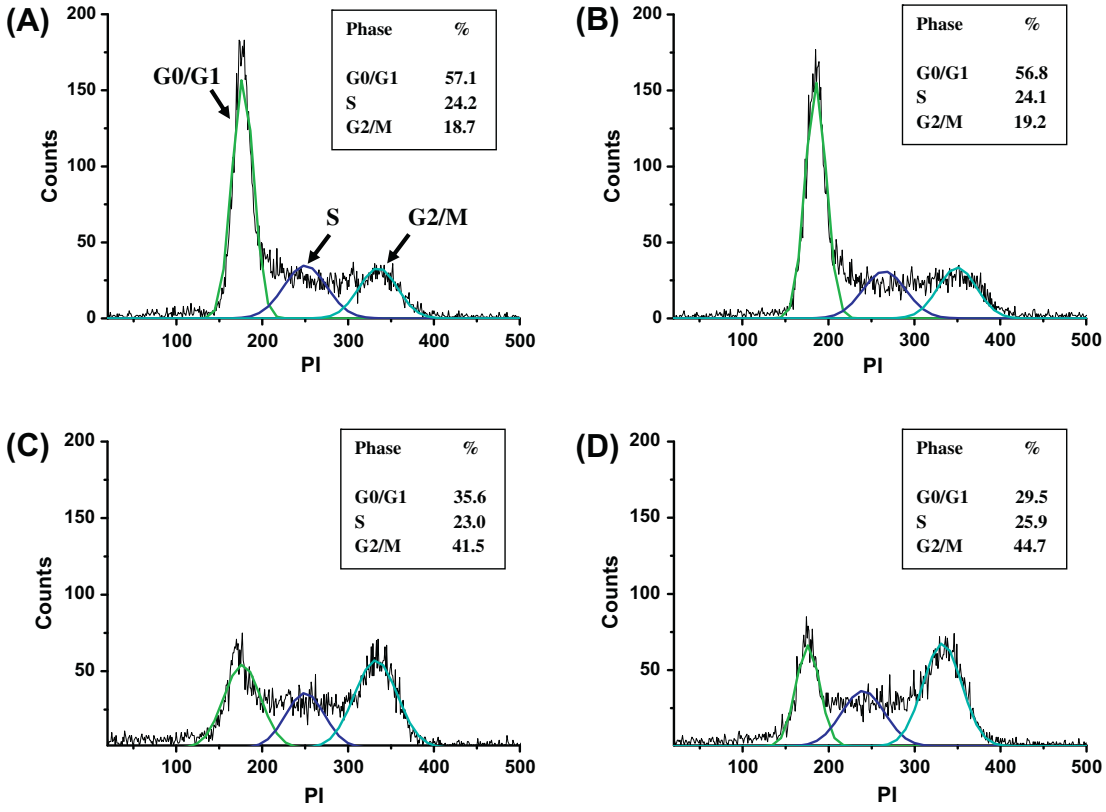

Fig. 8. Histograms of PI-associated K562 cell after 72-h incubation of control (A), with empty mPEG750-*b*-OCL-Bz micelles (B), with free QCT (C), and with QCT-loaded mPEG750-*b*-OCL-Bz micelles (D). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

also found for control cells (cells exposed to medium only), and this indicates that the empty mPEG750-*b*-OCL-Bz micelles had no effect on cell cycle progression.

In previous studies, it has been reported that QCT inhibits the growth of various human cancer cells and remarkably induces arrest in different phases of the cell cycle (in G2/M in breast cancer [40] and in G1/S in colon cancer cells [41]). The mechanism of QCT-induced cell cycle arrest in leukemia is still not clear. QCT arrested human leukemic T cells in late G1 phase [42], while human leukemic U937 cells were arrested in G2/M phase [15]. In the present study, the mechanism of leukemic K562 cell growth inhibition of QCT was found to be mediated through the inhibition of cell cycle progression with relatively high number of cells in the G2/M phase.

4. Conclusion

QCT was successfully loaded into mPEG750-*b*-OCL micelles with benzoyl or naphthyl capping groups prepared by a film hydration method and the solubility increased up to 1 mg/ml. Both micellar formulations showed a sustained release of the loaded QCT in about 24 h. The release from the benzoyl-modified micelles was slightly faster than that from the corresponding naphthoyl micelles. Furthermore, it was shown that empty benzyl-modified micelles showed good cytocompatibility. The QCT-loaded, benzyl-modified micelles had good cytotoxic effects against both resistant and normal cancer cells via inducing cell cycle arrest in the G2/M phase. Our results indicate that mPEG750-*b*-OCL micelles are an excellent solubilizer and an attractive vehicle for the delivery of QCT for cancer treatment.

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